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Effects of Myogenic Factor 5 (MYF5) Gene on Carcass and Meat Quality of Mong Cai Pigs

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Original Article

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Abstract

The aim of this study was to analyze the impact of Myogenic Factor 5 gene (MYF5) on carcass and meat quality of Mong Cai pigs. PCR-RFLP was applied to genotype 100 animals at three loci defined as MYF5/*Hin1*II, MYF5/*Hae*III and MYF5/*Msp*I. Results showed that MC pigs had two genotypes at each locus namely CD and DD, EF and EE, and GH and HH for the MYF5/*Hin1*II, MYF5/*Hae*III and MYF5/*Msp*I loci, respectively. The frequencies of DD, EE and HH were predominant (0.78-0.94) in the population. In addition, association analysis of three loci revealed that animals carrying CD genotype provided higher dressing percentage (70.30 vs. 67.32%) and loin weight (841 vs. 787 g) than those of DD genotype; however, these DD pigs had lower compression force value (4.91 vs. 5.79 kg). The other two polymorphisms were completely linked and no significant association was found with carcass and meat quality traits. Furthermore, the constructed haplotypes were identified to associate with hot carcass and dressing weight, of which the highest values were in CDEEHH pigs. These results suggested that the MYF5/*Hin1*II polymorphism was valuable for some carcass traits and compression force, but on the basis of this polymorphism, a simultaneous selection for these traits in MC pig remained a difficult task.

Keywords: carcass, pork quality, Vietnamese pigs

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บทคัดย่อ

ผลของยีน Myogenic Factor 5 (MYF5) ต่อคุณภาพซากและเนื้อของสุกรพันธุ์ Mong Cai

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จุดมุ่งหมายของการศึกษานี้เพื่อวิเคราะห์ผลกระทบของยีน Myogenic Factor 5 (MYF5) ต่อคุณภาพซากและคุณภาพเนื้อสุกร Mong Cai โดยใช้เทคนิค PCR-RFLP ในวิเคราะห์สายพันธุ์สัตว์จำนวน 100 ตัว โดยกำหนดตำแหน่งทั้งหมดสามตำแหน่ง ได้แก่ MYF5/*Hin*1II MYF5/*Hae*III และ MYF5/*Msp*I ผลการศึกษาพบว่าสุกร Mong Cai มีสองยีนในแต่ละที่คือ CD และ DD, EF และ EE และ GH และ HH สำหรับตำแหน่ง MYF5/*Hin*1II, MYF5/*Hae*III และ MYF5/*Msp*I ตามลำดับ โดยพบความถี่ของ DD, EE และ HH เป็นหลัก (0.78-0.94) จากกลุ่มประชากร นอกจากนี้การวิเคราะห์ความสัมพันธ์ของตำแหน่งทั้งสามแสดงให้เห็นว่าสุกรที่มีจีโนไทป์ชนิด CD มีเปอร์เซ็นต์ซากสุกร (70.30 เทียบกับ 67.32%) และน้ำหนักเนื้อสันหลัง (841 เทียบกับ 787 กรัม) สูงกว่าของจีโนไทป์ DD อย่างไรก็ตามสุกรที่มีจีโนไทป์ชนิด DD มีค่าแรงกด (compression force value) ที่ต่ำกว่า (4.91 เทียบกับ 5.79 กก.) สำหรับความหลากหลายอีกสองประเภทนั้นมีการเชื่อมโยงกันและไม่มีความสัมพันธ์อย่างมีนัยสำคัญคุณภาพซากและคุณภาพเนื้อสุกร นอกจากนี้พบ haplotype ที่สัมพันธ์กับน้ำหนักซากอุ่นและเปอร์เซ็นต์ซากสุกร ซึ่งมีค่าสูงสุดในสุกรกลุ่ม CDEEHH ผลการศึกษานี้ชี้ให้เห็นว่าความหลากหลายของ MYF5/*Hin*1II มีความสำคัญต่อคุณภาพซากบางประการและต่อค่าแรงกด แต่เมื่อคำนึงถึงความหลากหลายเหล่านี้การคัดเลือกเพื่อให้เกิดลักษณะร่วมกันในสุกร Mong Cai นั้นยังคงเป็นงานที่ยาก

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Introduction

Myogenic factor 5 (MYF5), a product of the MYF5 gene belonging to the MyoD family, plays an important role in the control of myogenesis and is responsible for the primary muscle fiber formation to their postnatal maturation and function (Hughes and Schiaffino, 1999). The MYF5 is related to the primary muscle cell migration and proliferation, especially for the satellite cell proliferation in the postnatal process of muscle regeneration (Pierzchała et al., 2008). This gene has been mapped to the porcine chromosome 5 (Soumillion et al., 1997), which contains three exons and two introns (te Pas et al., 1999). The expression and effects of MYF5 are different depending on breeds and it has been considered a candidate gene for meat production and meat quality (te Pas, 2004; Carmo et al., 2005). Because of its function involved in myoblasts proliferation, MYF5 gene was found to affect the proportion muscle fibers and the metabolic properties of muscle (Kłosowska et al., 2004). In cattle, MYF5 has been evidenced to influence the expression of muscle fiber types (Muroya et al., 2002) and thereby some meat quality traits (Ujan et al., 2011), while in pigs many studies have described the effect of MYF5 gene on carcass traits (te Pas et al., 1999; Cieślak et al., 2002; Liu et al., 2007[a]) but little is reported on its association with meat quality, especially in indigenous pig breeds. In Vietnam, Mong Cai (MC) is a popular local pig breed in the Central coastline, the Red River delta and other Northern provinces. Although it has slow growth rate, it has favored characteristics of good adaptation to poor-quality feed and superior meat quality traits (Ngu, 2006). The aim of this study was to reveal the association of MYF5 polymorphisms with carcass characteristics and meat quality traits obtained in MC pigs.

Materials and Methods

Animals and Sampling: This study was carried out on a hundred MC castrated male pigs, reared at a state farm in Quang Ninh province of Vietnam, at the body weight of 28.8±5.9 kg and at the age of 198±17.9 days. The pigs were fasted 12 hours before slaughtering following a commercial standard procedure and under the supervision of the veterinary service. Hot carcass weights were recorded and used for dressing percentage calculation. Longissimus dorsi (LD) muscle samples were collected for DNA extraction and meat quality evaluation. Meat color was determined using a Minolta Chromameter (CR310, Minolta, Japan) on a cut surface 24 hour post-mortem. Muscle pH was measured at 45 min ($pH_{45\ min}$) and 24 hour (pH_{24}) with a Delta-320 portable pH meter (Richmond Scientific Ltd.). Drip loss was calculated as the weight loss of a meat sample in an inflated bag at 4^0C for 24 hours (DL_{24}) and 48 hours (DL_{48}) (Honikel,

1998). For compression force values, samples were thawed at 4^{0}C and cut into 10 x 10 mm cross-sections and six samples for each loin were measured using a TA–XT2i Texture Analyzer (Stable Micro Systems Ltd) (Florowski et al., 2006). Meat chemical compositions such as the percentage of dry matter (DM), crude protein (CP) and ether extract (EE) of LD muscle were analyzed using standard AOAC methods (AOAC, 1998).

Genotyping: Total DNA was extracted using phenol-chloroform method. Primer sequences, restriction enzymes and PCR condition of the MYF5 gene (GenBank Acc. No.Y17154) were detailed by Liu et al. (2008). In brief, fragments were amplified in 20 µl reaction volume containing 25 ng genomic DNA as template, 0.25 µM dNTP, 0.25 µM of each primer, and 1 U Taq polymerase and PCR buffer. The reaction started with 4 min of initial denaturation at 94^{0}C, followed by 35 cycles of amplification with denaturation at 94^{0}C for 45 sec, annealing at 57^{0}C (MYF5-p1) or 63^{0}C (MYF5-p2) for 45 sec, extension at 72^{0}C for 60 sec, and a final elongation at 72^{0}C for 10 min. The PCR products of the MYF5-p1 and MYF5-p2 primers were subsequently used for genotyping by PCR-RFLP (Restriction Fragment Length Polymorphism) method. The three polymorphic sites, namely A1205C (exon 1), G2368A and A2165G (intron 1, exon 2, intron 2), were recognized by *Hin*1II, *Hae*III and *Msp*I, respectively. A total volume of 10 µl including 8.5 µl PCR product, 0.5 µl restriction enzyme and 1 µl reaction buffer was incubated at 37^{0}C for 4 hours. The digested products were checked and genotypes were directly determined by electrophoresis on a 2% agarose gel at 80 volt for 30 min. From the detected SNPs, a construction of haplotype using Merlin software (Abecasis et al., 2002) was performed and these haplotypes together with the SNPs were analyzed in the association study.

Statistical analysis: **A**ssociation analysis between MYF5 genotypes and traits of interest was performed using least squares method of the GLM procedure in Minitab version 13.20 with the following model: Yijk = µ + Gi + Cj + eijk, where Yijk was the observation of traits, µ was the overall mean of each trait, Gi was the genotype effect, Cj was the covariate (body weight at slaughter) and eijk was the random error. The difference between genotypes was tested using Turkey pair wise comparison at the 5% significance level. Data were presented as Least square means±standard error.

Results

Genotype and allele frequency: The PCR-RFLP data allowed identifying different genotypes at three polymorphic positions of the MYF5 gene. The electrophoresis results obtained from restriction enzyme fragments were: 243+210+33 bp for MYF5/*Hin*1II CD genotype; 210+33 bp for MYF5/*Hin*1II DD genotype; 1016+193 bp for MYF5/*Hae*III EE genotype; 1016+777+239+193 bp for MYF5/*Hae*III EF genotype; 575+461+173 bp for MYF5/*Msp*I HH genotype and 1036+575+461+173 bp for MYF5/*Msp*I GH genotype (Fig 1). The frequencies of genotypes and haplotypes of the SNPs (Single Nucleotide Polymorphism) are presented in Table 1. There was a similar trend of genotype distribution at all loci, in which the majority of animals carried DD, EE and HH genotypes (> 78%); however, there were only 2 genotypes found at each locus. The MYF5/*Hae*III and MYF5/*Msp*I were completely linked; their genotype frequencies for both mutations had the same values. Additionally, the most common haplotype was DDEEHH, followed by CDEEHH and DDEFGH.

Effects of polymorphisms on carcass and meat quality traits: Association analysis of the MYF5/*Hin*1II genotypes showed significant impacts of CD and DD genotypes on dressing percentage and loin weight ($p < 0.05$) (Table 2). Moreover, the pH45 min value was detected to be higher in DD compared to CD pigs ($p < 0.05$). The DD genotype had significantly lower compression force value than CD genotype ($p < 0.05$). For the other two SNPs, significant associations between MYF5/*Hae*III and MYF5/*Msp*I genotypes with carcass and meat quality traits were not detected (data not shown). In addition, MYF5 haplotypes were significantly associated with hot carcass and dressing percentage ($p < 0.05$) with highest values in animals carrying CDEEHH haplotype, whereas no differences were apparent for other meat quality traits (Table 3). There was also a trend of increased loin weight in CDEEHH pigs with the significance of $p = 0.052$. At all loci examined, statistical effects of either genotypes or haplotypes on meat chemical composition (Table 2 and Table 3) were not established ($p > 0.05$).

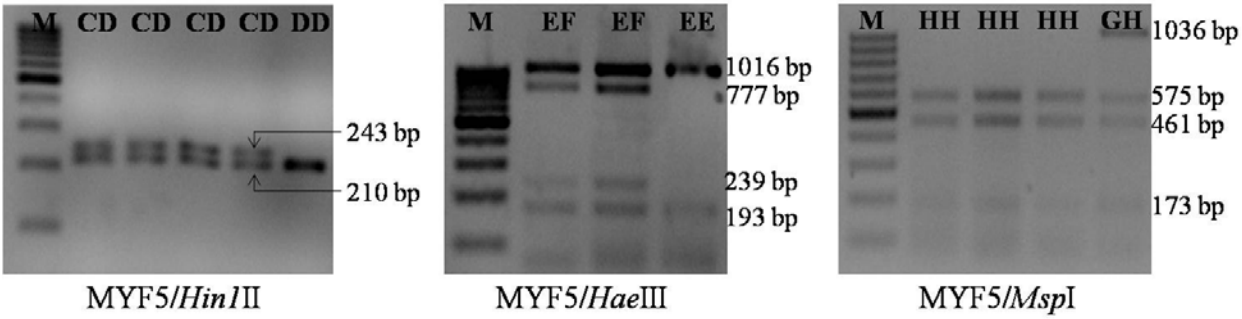

Figure 1 Agarose gel electrophoresis of PCR-RFLP at MYF5/*Hin*1II, MYF5/*Hae*III and MYF5/*Msp*I loci. M: 100 bp DNA ladder, Fermentas.

Table 1 Genotype and haplotype frequencies of MYF5 gene in MC pigs

Item	MYF5/*Hin*1II		MYF5/*Hae*III		MYF5/*Msp*I		MYF5 haplotype		
	CD	DD	EF	EE	GH	HH	DDEEHH	CDEEHH	DDEFGH
No. of pigs	22	78	6	94	6	94	73	21	5
Frequency	0.22	0.78	0.06	0.94	0.06	0.94	0.74	0.21	0.05

Table 2 Association of MYF5/*Hin*1II genotypes and meat quality traits in MC pigs

Traits	Genotype		*p*-value
	CD (n = 22)	DD (n = 78)	
Carcass			
Dressing percentage (%)	70.30 ± 0.97	67.32 ± 0.53	0.009
Loin weight (g)	841.4 ± 22.23	786.9 ± 12.11	0.039
Meat quality			
$pH_{45\ min}$	6.53 ± 0.05	6.65 ± 0.02	0.018
pH_{24}	6.09 ± 0.04	6.12 ± 0.02	0.530
Drip $loss_{24}$ (%)	1.98 ± 0.16	1.79 ± 0.09	0.499
Drip $loss_{48}$ (%)	2.87 ± 0.20	2.58 ± 0.11	0.237
Cooking loss (%)	23.35 ± 0.89	22.21 ± 0.49	0.264
Compression force (kg)	5.79 ± 0.37	4.91 ± 0.19	0.034
Meat color			
*L** (Lightness)	48.94 ± 0.34	49.29 ± 0.19	0.353
*a** (Redness)	4.97 ± 0.60	5.59 ± 0.32	0.369
*b** (Yellowness)	8.79 ± 0.31	8.92 ± 0.17	0.997
Meat chemical composition (%)			
Dry matter	25.45 ± 0.30	25.63 ± 0.16	0.946
Crude protein	21.99 ± 0.22	22.09 ± 0.12	0.612
Ether extract	2.42 ± 0.11	2.58 ± 0.06	0.148
Ash	0.94 ± 0.08	1.01 ± 0.04	0.238

Table 3 Association of MYF5 haplotypes and carcass and meat quality traits in MC pigs

Traits	Haplotype			*p*-value
	DDEEHH (n = 74)	CDEEHH (n = 21)	DDEFGH (n = 5)	
Carcass				
Dressing percentage (%)	67.44 ± 0.53^{b}	70.64 ± 0.97^{a}	68.01 ± 2.02^{ab}	0.016
Loin weight (g)	792.0 ± 12.17	847.9 ± 22.39	753.4 ± 46.78	0.052
Meat quality				
$pH_{45\ min}$	6.63 ± 0.03	6.55 ± 0.04	6.69 ± 0.10	0.261
pH_{24}	6.09 ± 0.02	6.10 ± 0.04	6.15 ± 0.09	0.423
Drip $loss_{24}$ (%)	1.83 ± 0.11	2.12 ± 0.21	1.73 ± 0.44	0.433
Drip $loss_{48}$ (%)	2.57 ± 0.13	2.94 ± 0.23	2.55 ± 0.50	0.388
Cooking loss (%)	22.40 ± 0.51	22.41 ± 0.96	21.57 ± 1.99	0.920
Compression force (kg)	4.95 ± 0.20	5.17 ± 0.38	5.50 ± 0.79	0.715
Meat color				
*L** (Lightness)	49.37 ± 0.19	48.87 ± 0.34	48.62 ± 0.71	0.287
*a** (Redness)	5.72 ± 0.33	4.89 ± 0.60	7.76 ± 1.26	0.110
*b** (Yellowness)	8.94 ± 0.17	8.71 ± 0.32	8.80 ± 0.66	0.804
Meat chemical composition (%)				
Dry matter	25.43 ± 0.17	25.49 ± 0.30	25.25 ± 0.63	0.937
Crude protein	21.94 ± 0.12	22.27 ± 0.22	21.92 ± 0.46	0.404
Ether extract	2.52 ± 0.06	2.37 ± 0.12	2.70 ± 0.24	0.367
Ash	1.04 ± 0.05	0.86 ± 0.08	1.01 ± 0.17	0.173

$^{a, b}$ Values in the same row with different superscripts are significantly different ($p < 0.05$)

Discussion

Our study confirms the findings in many pig breeds that the presence of homozygous genotypes CC, FF and GG was very rare and by investigating the association of three SNPs on carcass and meat quality traits, only the MYF5/*Hin*1II was observed to influence dressing percentage, loin weight, meat pH and compression force.

In the current study, the MYF5/*Hin*1II polymorphism provided two genotypes with the majority being the DD animals. This partly supported the report of Liu et al. (2007[b]) that in a commercial population of Yorkshire, Landrace and their cross, this locus was polymorphic for the D allele whereas

three genotypes were detected in the Meishan, Yorkshire and Meishan x Yorkshire cross. At the MYF5/*Msp*I locus, the GG genotype could not be found and the HH genotype was predominant in both Large White and Landrace breeds (Verner et al., 2007). In the LW x Meishan F2 population, a small number (5.1%) of GG animals was also described by Liu et al. (2007[a]). This was later validated by Liu et al. (2008), who found no GG genotype in the Large White and Landrace, whereas in the Meishan breed the GG and GH frequencies were 0.18 and 0.38, respectively, which was higher than those of MC pigs (0 and 0.06, respectively). Thus, irrespective of breed, the G allele was in low frequency across populations.

The first polymorphism in exon 1 of the MYF5 gene resulted in amino acid substitution (Met → Leu), which may be responsible for changing the functional properties of the protein and affecting muscle maturity (Liu et al., 2008). In the present study, the analysis of MYF5/*Hin*1II polymorphism with carcass traits indicated that pigs with genotype DD had significantly lower dressing percentage and loin weight as compared to those with genotype CD. In the Large White x Meishan F2 population, Liu et al. (2007[b]) observed significant associations between this locus with fat deposition traits and carcass length, in which the difference mainly between the CC and DD animals implied that allele "C" was closely related to higher back fat and buttock fat thickness content. Although CC animals were not available in this study, it could be that the impacts were breed-specific due to different genetic backgrounds and selection pressure or it might originate from the difference in muscle fiber type proportion, where higher percentages of IIx and IIa were found in MC pigs compared to IIb fibers (data not shown). It also suggested that this mutation could not be causal for the differences in carcass traits being investigated. The haplotype analysis, of which the lowest carcass values in pigs bearing DDEEHH variants, additionally confirmed the data.

The MYF5 gene was reported to have a relationship with the expression of fast-twitch oxidative fiber (Kłosowska et al., 2004) and thus it is likely to have further impact on metabolic activity of the muscle and meat quality. Regarding to this point, for the MYF5/*Hin*1II SNP, Liu et al. (2008) demonstrated changes of intramuscular fat and meat moisture content with the substitution of amino acid caused by this polymorphism. However, these outcomes were not confirmed in this study, showing only significant effects on $pH_{45\ min}$ and compression force of the meat. In the present work, although there was a discrepancy of compression force values between two genotype groups, the intramuscular fat reflected by EE value was similar. According to the observations of Florowski et al. (2006) on a Polish indigenous pig breed, the relationship between compression force and intramuscular fat content was negative (-0.36), and thus higher fat deposition would be expected in homozygous DD pigs. The non-significant difference was probably because of the limited animals examined, especially the imbalance among the genotypes. In combination with the results of the association with carcass, it can be stated that a simultaneous selection for both better carcass and meat quality in MC pig based on this polymorphism would be a difficult task to accomplish. As there were only a few animals bearing the EF and GH genotype, a wider study with larger numbers and in other native breeds would be of interest for analysis.

In conclusion, among the investigated MYF5 polymorphisms, the MYF5/*Hin*1II locus is of valuable SNP that has certain impacts on $pH_{45\ min}$, meat compression force in LD muscle, dressing percentage and loin weight. Further studies are needed to confirm the association in other native populations before applying this gene as a genetic marker for pig breeding selection.

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